REMARKS

Status of the Claims

Claims 1-22 are pending in this application and stand rejected. After entrance of this amendment, claims 1, 8, 13 and 21 are amended. No new matter is introduced by these amendments.

Sequence Compliance

Acknowledgement of compliance with the sequence listing requirements and entry of the CFR is noted with appreciation.

Amendment to the Specification

The objection to the Abstract for use of the word "said" is noted and applicants have amended the Abstract, *inter alia*, to remove the use of the word "said". No new matter is introduced by these amendments.

Claim Rejections – 35 U.S.C.§112

Claims 1-22 are rejected under 35 U.S.C.§112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. In particular, the Office Action states the use of the phrase "substantially chemically identical" renders claims 1 and 13 vague and indefinite. Furthermore, the Office Action states there is an ambiguity with respect to claims 1 and 13 because it is not clear if the proteins are reacted with both reagents of the set or merely one. Regarding claims 8 and 21, the Office Action states that the phrase "including" makes it unclear as to whether the limitation following the phrase are part of the clamed invention and that the Markush format is improper due to the use of the conjunction "or" instead of the recommended conjunction "and".

It is submitted that the claim amendments presented herein overcome this ground of rejection and that the claims as amended now meet the requirements of 35 U.S.C.§112.

Claim Rejections – 35 U.S.C.§103

Claims 1-3, 6-8, and 10-21 are rejected under 35 U.S.C.§103(a) as allegedly being unpatentable over Aebersold et al. (WO 00/11208) ("Aebersold") in view of Sechi et al. (Analytical Chemistry, 1998, 70, 5150-5158) ("Sechi"). Claims 4 and 5 are rejected as being unpatentable over Aebersold in view of Sechi and in further view of Yates et al. (US Patent No. 5,538,897 ("Yates"). Claim 9 is rejected as being unpatentable over Aebersold in view of Sechi and in further view of Bienvenut et al. (Analytical Chemistry, 1999, 71, 4800-4807) ("Bienvenut"). Claim 22 is rejected as being unpatentable over Aebersold in view of Sechi and in further view of Clauser et al. (Proceedings of the National Academy of Sciences, USA, 1995, 92(11), 5072-6) ("Clauser").

The Office Action asserts that Aebersold teaches methods of analyzing proteins or protein function in complex mixtures and further asserts such methods use labeling compositions comprising the formula A-L-PRG wherein A represents an affinity label, PRG is a protein reactive group and L is a linker group. The Office Action concludes that Aebersold differs from the present invention in not specifically including gel electrophoresis as a protein separation step in their method. This deficiency is allegedly cured by the teachings of Sechi.

First, applicants' invention represents an improvement over Aebersold by providing the capability to analyze proteins and protein function through a more introspective examination of the proteome by reducing the complexity of a complex protein mixture and, in certain instances, of a peptide mixture. This is accomplished by first separating the labeled proteins prior to proteolytic digestion and then, if desired, the resultant peptide mixture can be separated through affinity selection. Identification of separated proteins from a complex mixture, such as proteins contained in sera or cell/tissue lysates and the like can be made with more confidence when a pre-digestion separation step is employed than with respect to unseparated mixtures. While Aebersold employs a composite reagent that is similar to one species used by applicants to label two or more protein samples, Aebersold's methodology is totally different from that set forth by applicants and produces results in certain analyses that are determined with less confidence than with the method of the present invention. Applicants' claims require an electrophoresis step, such as gel electrophoresis, prior to digestion to separate individual proteins in the mixture, thereby significantly reducing sample complexity. Aebersold does not disclose, suggest or

motivate one to use electrophoresis as a separation technique, let alone to employ such separation mechanism immediately after labeling the protein samples. Instead, Aebersold (after labeling the protein samples) describes starting the analysis at the lysate level and digesting the proteins in the sample after labeling. Thereafter Aebersold uses an affinity step to separate cysteine-containing peptides from the mixture. Thus Aebersold starts with a complex mixture and carries that complexity throughout the workflow until much later in the methodology when an affinity separation step is used. While some level of reduced complexity is achieved with the methods of Aebersold, it is only realized at the affinity step. It is not possible with the use of an affinity step to distinguish peptides and the proteins from which they are derived. By first separating the proteins using electrophoresis prior to digestion as required in applicants' claims, proteins can be identified with greater confidence since the interfering peptides derived from most of the other proteins are removed from the mixture. Spatial orientation (i.e., isoelectric point (pI) and apparent molecular weight (MW)), for example as preserved in a 2-D gel, provides additional information that can be used to increase the confidence level of protein identification, and the ability to use such added information is not described or suggested by Aebersold.

As mentioned, there is no motivation or even remote suggestion in Aebersold to employ electrophoresis in their methods. It is well settled that to combine references to establish a *prima facie* case of obviousness, the motivation for the combination must come from the references themselves. Thus applicants respectfully disagree with the Office Action's assertion that it would have been obvious to one of ordinary skill in the art to utilize electrophoresis separation as taught by Sechi to identify and separate proteins from a complex mixture as taught by Aebersold.

Assuming for sake of argument that there were sufficient motivation to combine the teachings of Aebersold and Sechi, it is submitted that such combination would not be operable as described and claimed in applicants' invention. Sechi describes the use of cysteine alkylation as being required prior to electrophoresis to avoid formation of acrylamide adducts during electrophoresis. Sechi does not compare protein compositions between two or more different protein samples, rather Sechi takes a single protein sample, splits the sample in two and labels this split sample with either deuterated or non-deuterated acrylamide. The alkylation technique taught by Sechi (wherein acrylamide was found to produce the most efficient alkylation) is simply to enhance the confidence level of being able to identify cysteine-containing peptides of a single protein. Isotope labeling as taught by Sechi is used to discriminate and locate peptides

that have been derivatized with acrylamide. Sechi also teaches that the labeling with acrylamide permits the number of cysteines contained in a peptide to be determined in a MALDI-MS peptide mass fingerprinting analysis. Sechi does not describe or remotely suggest labeling of cysteine-containing proteins to perform comparisons of two different protein samples, such as for protein expression analysis by determining relative quantification between two or more protein samples in a protein analysis experiment. Thus, it is submitted that Sechi does not cure the deficiencies of Aebersold.

In view of the reasoning set forth above with respect to the Aebersold and Sechi references, the rejections of claims 4 and 5, 9 and 22 should likewise be withdrawn. First, each of claims 4, 5, 9 and 22 depends from claim 1, and the rejections to these claims have been made by combining a third reference with Aebersold and Sechi. Applicants submit that they have demonstrated that neither Aebersold nor Sechi, taken alone or in proper combination, render the claims as being unpatentable. Therefore, applicants respectfully request reconsideration of the specific rejections to claims 4, 5, 9 and 22.

Applicants submit that the present invention is patentable over the prior art because the prior art fails to describe or suggest a method of comparing protein compositions between two or more samples by providing a set of differentially isotopically labeled protein reagents, reacting each set of protein sample with one of the proteins reagents to provide isotopically, but differentially labeled protein samples, mixing the two protein samples, electrophoresing the mixture prior to protein digestion and detecting the difference in protein expression levels by mass spectrometry.

CONCLUSION

In view of the arguments presented herein, applicants respectfully submit that claims 1-22 are in condition for allowance. It is respectfully requested that the outstanding rejections of the pending claims be reconsidered and withdrawn, and a Notice of Allowance is courteously requested. If the Examiner believes a telephone conversation would be helpful to advance the prosecution of the present application, she is invited to call the undersigned Attorney for Applicants at (508) 383-7406.

Respectfully submitted,

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